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**FATE OF PENTABORANE-9-H³ IN SMALL ANIMALS AND
EFFECTS OF PENTABORANE-9 UPON GLUCOSE CATABOLISM
BY RATS**

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FOREWORD

This study was initiated by the Biomedical Laboratory of the Aerospace Medical Research Laboratories, Aerospace Medical Division, Wright-Patterson Air Force Base, Ohio. The research was performed in support of Project No. 6302, "Toxic Hazards of Propellants and Materials," Task No. 630202, "Pharmacology and Biochemistry," under Contract No. AF 33(657)-11757, with the Science Research Institute, Oregon State University, Corvallis, Oregon. Dr. C. H. Wang was the principal investigator for Oregon State University and A. A. Thomas, MD and K. C. Back, PhD were contract monitors for the Toxic Hazards Branch, Physiology Division. Research was initiated 1 June 1963 and completed 30 June 1964.

The technical assistance of Mr. Royal D. Barbour in this work is greatly appreciated.

This technical report has been reviewed and is approved.

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ABSTRACT

The fate of pentaborane-9- H^3 (B_5H_9^3 , evenly labeled with tritium) in small animals was investigated. Rats given liquid B_5H_9^3 by intraperitoneal injection evolved 36-37% of the tritium label as molecular hydrogen over a 2-3 hour period. A non-volatile hydrolysis intermediate rapidly formed in the bloodstream concurrently as molecular hydrogen was evolved by treated animals. Tritium in the hydrolysis intermediate was found to be non-exchangeable into water. However, the hydrolysis intermediate slowly disappeared and approximately an equivalent amount of tritium could be detected in the body water of treated animals. Reaction of heparinized blood and certain nitrogen-containing compounds with B_5H_9^3 resulted in the formation of a hydrolysis intermediate which had many properties similar to those of the stable intermediate formed in vivo. The effect of pentaborane-9 intoxication upon glucose catabolism by rats was examined. Total respiratory CO_2 production by intoxicated rats was slightly greater than that of normal rats. Catabolism of glucose via the glycolytic pathway appeared to be inhibited during the initial 4 hours after B_5H_9 administration by intraperitoneal injection. The operation of the pentose phosphate pathway may have been stimulated slightly immediately after B_5H_9 administration, but inhibition of this pathway was noted during the next several hours of glucose assimilation. Pentaborane-9 intoxication appears to affect glucose catabolism for only a 6-8 hour period.

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FATE OF PENTABORANE-9- H^3 IN SMALL ANIMALS

SECTION I

INTRODUCTION

The toxicity of boron hydrides has been investigated by several workers (ref. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 & 13). Typical symptoms of pentaborane-9 (B_5H_9) intoxicated human patients include; light headedness, dizziness, headaches, loss of memory, lack of coordination, tremors and muscle spasms (ref. 8). With small animals, it has been reported that pentaborane-9 causes hyperactivity of the central nervous system (ref. 11 & 12). Possible correlations between the reducing power of the boranes and their respective toxicity, has been studied by Hill (ref. 2). However, defined conclusions could not be reached since the boranes appear, in some cases, to react with water prior to its interaction with the reagent, phosphomolybdic acid. Miller, et al (ref. 7) has reported that a linear relationship exists between serum borane levels and toxic symptoms of the test animals upon exposure to decaborane or its derivative, HEF-3. These findings, consequently, indicate that the toxic actions of pentaborane-9 may be related to its reducing power or to its ability to form relatively stable complexes with biological compounds.

In the present work, the fate of pentaborane-9 and its degradation products in small animals has been studied by means of the radiotracer method, employing pentaborane-9 evenly labeled with tritium ($B_5H_9^3$). Emphasis has been placed on the mechanism of hydrolysis of pentaborane-9 in biological systems, leading to the formation of partially hydrolyzed product(s). Also studied was the transfer of hydrogen atoms of pentaborane-9 to other biological constituents. The experimental findings obtained in this work have been correlated with previous toxicological studies on pentaborane-9 in small animals (ref. 1).

SECTION II

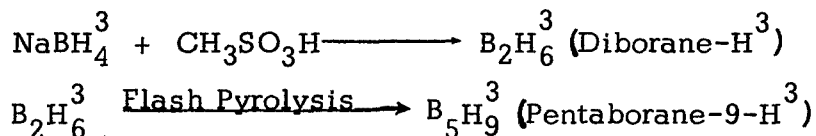
MATERIALS

Experimental Animals

The rats used in the experiments described in this report were male Sprague-Dawley rats obtained from Pacord Research, Inc., Portland, Oregon. The rabbits used were cross breeds of local origin. The animals were maintained on Purina Laboratory Chow with water ad libitum prior to the experiments. In experiments requiring sacrifice of the animals, sodium pentobarbital euthanasia was employed.

Pentaborane-9-H³

Evenly labeled pentaborane-9-H³, with a specific activity of 195 $\mu\text{C}/\mu\text{l}$, was prepared by New England Nuclear Corporation, Boston, Massachusetts, according to the methods of Weiss and Shapiro (ref. 14), McCarthy and Di Giorgio (ref. 15) and Owen (ref. 16). The reactions used in synthesis were the following:



SECTION III

METHODS

Pentaborane-9-H³ Administration

Pentaborane-9-H³ was vacuum transferred into a glass dispenser according to the method previously reported by this laboratory (ref. 1). Liquid pentaborane-9 administration to the animals was by intraperitoneal injection with a 10 μl microsyringe. (Hamilton Company, Whittier, California.)

Tritium Assay Methods

All tritium assays were carried out in duplicate or triplicate and data presented are averages of these assays. Counting was carried out over a sufficient length of time so that the relative standard deviations were less than 2%. All samples for tritium assay were counted in a Packard liquid scintillation spectrometer (Model 314-EX).

Assay for Tritium in Water

A 0.01-0.10 ml aliquot of water was mixed with 5 ml of absolute ethanol and 10 ml of scintillation solution. The scintillation solution consisted of 10 ml toluene containing 3 grams terphenyl and 30 mg POPOP (1,4-bis-2-(5-phenyloxazolyl)-benzene) per liter.

Assay for Tritium in Dried Blood and Tissue

Aliquots (10-20 mg) of dried blood and ground tissues were oxidized to CO₂ and water by the Schöniger combustion technique (ref. 17). The water formed from the combustion was mixed with 10 ml ethanol and a 1-5 ml aliquot removed and mixed with 10 ml of scintillation solution for H³ assay.

Assay for Tritium in Fresh Tissue

Small portions of the organs and tissues after excision were placed immediately in gelatin capsules and frozen on dry ice. The frozen samples were oxidized to CO_2 and water by an oxygen bomb technique, similar to that reported by Sheppard and Rodegker (ref. 18). The water of combustion was mixed with absolute alcohol and scintillation solution for tritium assay.

Assay for Tritium in Pentaborane-9- H^3

Microliter amounts of pentaborane-9- H^3 were dissolved in anhydrous scintillation solution. Aliquots of this solution were added to additional anhydrous scintillation solution and assayed directly in a liquid scintillation spectrometer. Anhydrous scintillation solution was prepared by drying the solution over freshly cut sodium metal.

Molecular Tritium Formation by Pentaborane-9- H^3 Treated Rats

Two rats weighing 250-260 grams each were administered liquid B_5H_9^3 (sp.act. $6.5 \mu\text{C}/\mu\text{l}$) at a dose level of 2.5 mg/kg by intraperitoneal injection. Each rat was placed immediately in an individual respiration chamber of an animal respirometer system. The animal respirometer, which has been described in another report (ref. 19) consisted of individual respiration chambers, Drierite drying columns, flow ionization chambers, vibrating reed electrometers, and an analog-to-digital readout system. Previous experiments had shown that all of the evolved radioactivity in the expired respiratory gases was in the form of tritium, except for a trace amount of tritiated water which was removed by the drying column.

Changes in Tritium Distribution in Rabbit Blood (In Vivo) vs. Time After B_5H_9^3 Administration

A heparinized and anesthetized rabbit weighing 840 grams was administered $3.7 \mu\text{l}$ of liquid pentaborane-9- H^3 ($1.5 \mu\text{C}/\mu\text{l}$) intraperitoneally by means of a microsyringe. Blood samples (0.5 ml) were drawn at prescribed intervals (figure 3) by means of an indwelling cannula in an ear vein. The blood samples were frozen immediately in a dry-ice cooling bath and then lyophilized. The water of lyophilization was counted in a liquid scintillation counter as described. A portion of the dried blood was oxidized to CO_2 and water according to the Schöniger combustion method described.

Tritium Distribution in Rat Tissues Ten Minutes After B_5H_9^3 Administration

A male rat weighing 250-260 grams was administered $4.4 \mu\text{l}$ of B_5H_9^3 ($850 \mu\text{C}$) via intraperitoneal injection. Ten minutes later, the animal was sacrificed and the main organs and an aliquot of blood removed. These tissues were frozen immediately in a liquid-nitrogen cooling bath. They were then finely ground in a pre-cooled mortar (liquid nitrogen) with a pestle.

The ground tissues were individually lyophilized to obtain dried tissues and water as separate fractions for tritium assay. Tritium assays were performed as described.

Tritium Distribution in Rat Tissues Three Hours After $B_5H_9^3$ Administration

Male Sprague-Dawley rats weighing approximately 350-400 grams were injected intraperitoneally with pentaborane-9- H^3 (sp.act. 195 $\mu\text{C}/\mu\text{l}$) and placed in the animal respirometer. The rats were sacrificed, after molecular tritium evolution had essentially ceased (3 hours) and their organs and tissues were removed for tritium assay. Small portions of the organs and tissues were placed immediately in gelatin capsules, frozen in dry ice, and tritium content determined by the oxygen bomb technique described. The data shown in table III is an average of two or more analyses. The tritium recoveries are expressed on both a total organ and gram fresh weight basis.

Tritium Distribution in Rat Blood Three Hours After Administration of $B_5H_9^3$

Two rats, weighing 300 grams each, were administered pentaborane-9- H^3 , 1.05 and 1.8 μl (195 $\mu\text{C}/\mu\text{l}$) respectively, by intraperitoneal injection. The rats were sacrificed 3 hours later and blood samples were drawn by heart puncture. The blood samples were lyophilized and assayed for tritium in a manner identical to that for the rabbit blood samples.

Interaction of Pentaborane-9- H^3 with Blood In Vitro

The in vitro reaction of heparinized rat blood with pentaborane-9- H^3 was studied by two methods: (1) Blood was placed in a one-liter glass bulb, equipped with two teflon stopcocks, and rotated slowly. The bulb was first flushed with nitrogen at atmospheric pressure to prevent the reaction of pentaborane-9- H^3 with oxygen from air. Pentaborane-9- H^3 was injected into the bulb via one of the teflon stopcocks previously equipped with a rubber serum stopper isolated from the bulb atmosphere with a small amount of mercury. After the pentaborane-9- H^3 injection was made with a microsyringe, the stopcock was closed to prevent the mercury from being mixed with the pentaborane-blood mixture as the bulb was slowly rotated by a rotary evaporator drive motor. (2) The second method for exposing blood samples to pentaborane-9- H^3 was by the use of a high vacuum manifold system (Delmar Scientific Laboratories, Maywood, Illinois). The reaction system was a portion of the high vacuum system and consisted of a 100 ml reaction flask and a mercury manometer. It was isolated from the remaining vacuum manifold system by mercury float valves. By use of the mercury manometer it was possible to follow the rate of molecular hydrogen evolution during the course of the blood-pentaborane-9 reactions. The reactions were carried out in vacuo at room temperature ($24^\circ\text{C} \pm 2^\circ\text{C}$) and stirred with a magnetic stirrer.

In a typical reaction, 5 ml of fresh heparinized rat blood was placed in the reaction flask. The reaction flask was then attached to the vacuum

system. Next, the reaction mixture was frozen by a liquid nitrogen bath, evacuated, and approximately 0.3 mmole of pentaborane-9- H^3 (sp.act. 96 μC /mmole) was vacuum transferred onto the frozen blood. After thawing, the reaction mixture was allowed to warm to room temperature and was maintained at room temperature for the desired length of time. At the end of the reaction period, the reaction mixture was re-frozen in a liquid nitrogen cooling bath. The molecular hydrogen was then removed by a Toepler pump and measured manometrically. The frozen blood-pentaborane-9- H^3 reaction mixture was then lyophilized and the water of lyophilization and the blood residue, or solids, were analyzed separately for tritium content.

Examination of the Blood-Pentaborane-9- H^3 Reaction Mixtures: Fractionation with Trichloroacetic Acid (TCA)

Heparinized rat blood was exposed to microliter amounts of pentaborane-9- H^3 by the two methods previously described. The flow schemes shown in Figures 4 and 5 illustrate two methods of TCA fractionation to determine tritium distribution in blood constituents. The fractionation method illustrated in figure 5 is essentially that of Bligh and Dyer (ref. 20). All tritium assays were performed directly on aliquots of any aqueous phases and Schöniger flask combustions on any residue or protein-containing fractions.

Hydrolysis of Pentaborane-9- H^3

The hydrolysis of pentaborane-9- H^3 by various solvents and nitrogen containing compounds was carried out in vacuo. The system and procedures were identical to those employed for reaction of blood with pentaborane-9 in the vacuum manifold system.

SECTION IV

RESULTS AND DISCUSSION

Molecular Tritium Formation by Pentaborane-9- H^3 Treated Rats

Figure 1 shows the interval recoveries (5 minute intervals) of molecular tritium (H_2^3) as percent of the radioactivity of $B_5H_9^3$ administered to rats. The rate of H_2^3 appearance in the expired air was very rapid initially and reached a maximum rate about 15-20 minutes after $B_5H_9^3$ administration. The second peak of H_2^3 formation at 35-40 minutes after $B_5H_9^3$ administration is interpreted as representing further hydrolysis of a partial hydrolysis product of $B_5H_9^3$. From this, it appears that the initial hydrolysis step was followed by a slower second step in the hydrolysis or metabolism of pentaborane-9 by intact rats. A very interesting aspect of the in vivo hydrolysis of this compound was the termination of molecular tritium release at a point far from completion (i.e., approximately 37% of H_2^3 production relative to the theoretical maximum yield) by 2 hours after $B_5H_9^3$ administration (figure 2). The experiments

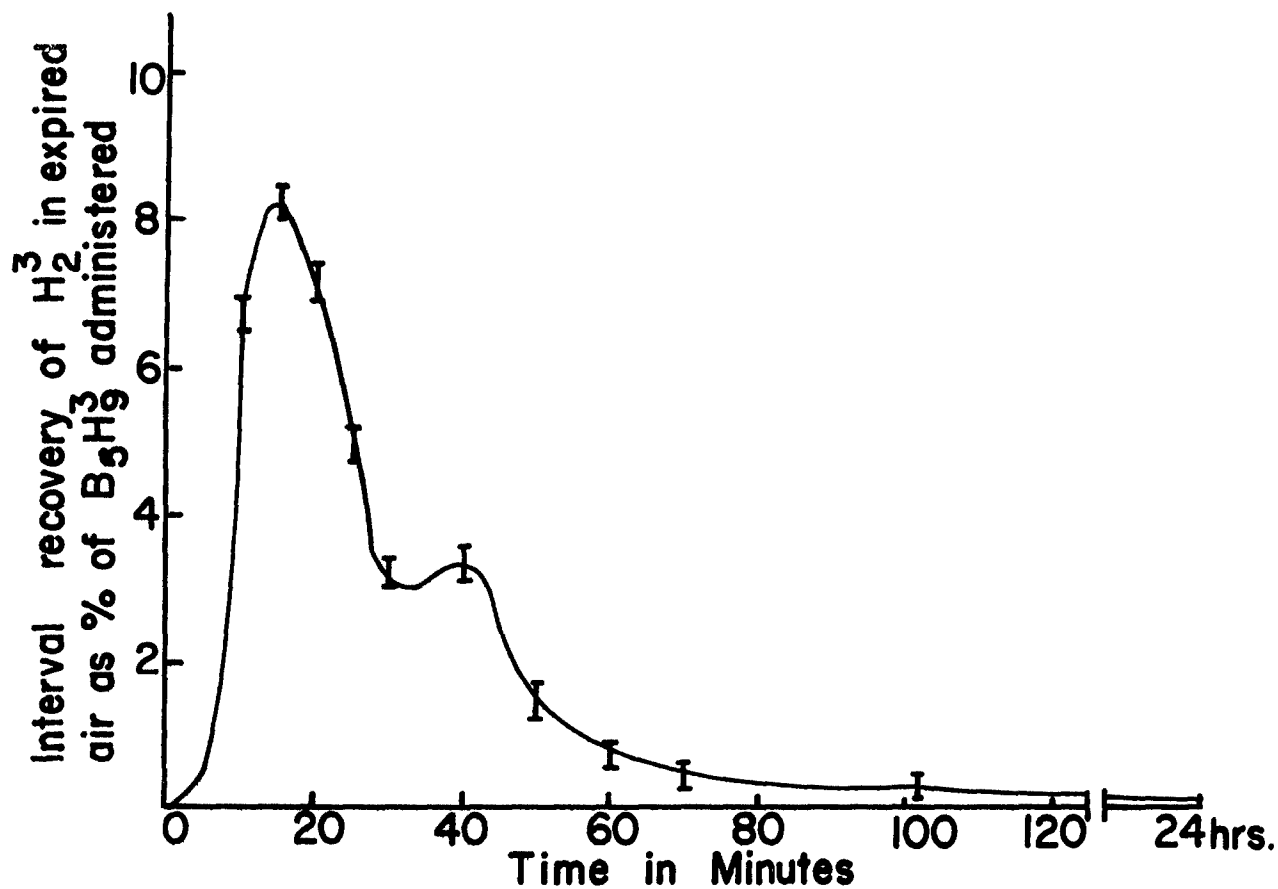


Figure 1. The formation of H_2^3 from rats given pentaborane-9- H^3 by intraperitoneal injection. Bracket points are from two separate experiments and are shown as individual values. The percentages are plotted on an interval recovery versus time basis. Five minute intervals were employed.

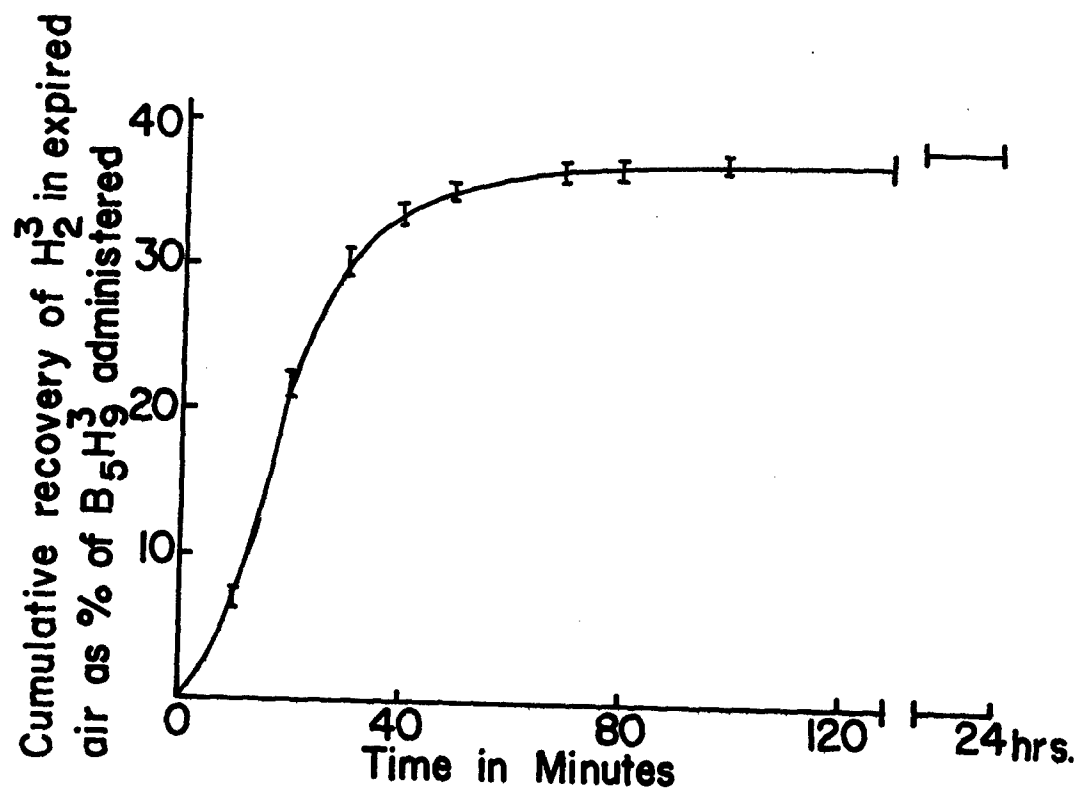


Figure 2. The formation of H_2^3 from rats given pentaborane-9- H^3 by intraperitoneal injection. The experimental data are those given in figure 1 but plotted on a cumulative basis.

were conducted for 24 hours and during the period from 2-24 hours after $B_5H_9^3$ administration, only a very small amount of molecular tritium was released in the expired air from the experimental animals.

In order to determine that H_2^3 release was not occurring at the site of injection (peritoneal cavity) rats were sacrificed by a sharp blow on the neck, injected immediately with pentaborane-9- H^3 and placed in the animal respirometer. It was found that the rate of molecular tritium release was extremely slow, even less than the rate of H_2^3 release from the live, intact rats during the slow phase, i.e., after the initial 2 hours. From this it can be concluded that the major portion of $B_5H_9^3$ hydrolysis or metabolism must occur after this compound reaches the animal's blood stream. Also, the initial high rate of hydrolysis was not simply an interaction between pentaborane-9- H^3 and the fluids and membranes of the peritoneal cavity.

To better understand the fate of the tritium atoms of $B_5H_9^3$ which were not evolved as molecular tritium, experiments were carried out to examine the nature of the $B_5H_9^3$ residue in the blood.

Tritium Distribution in Rabbit Blood Constituents vs. Time After $B_5H_9^3$ Administration

Shown in figure 3 and table I is the incorporation of tritium radioactivity into the blood constituents of a rabbit which was administered $B_5H_9^3$ via intraperitoneal injection. Tritium labeling of the dried blood fraction occurred at a very rapid rate for the first 13-14 minutes and then decreased almost as rapidly. The tritium radioactivity present in the blood as a non-volatile compound did not appear to contain ionizable tritium labeling, since the incorporated radioactivity failed to undergo an exchange reaction¹ with water to any appreciable degree.

A comparison of the rate of formation of the non-volatile tritium-labeled compound in the blood stream of exposed rabbits with the rate of molecular tritium evolution from the rat is of considerable interest. It can be seen from figures 1 and 3 that molecular tritium evolution and the formation of non-volatile tritium-labeled compounds in the blood occurred simultaneously. The detected peaks of molecular tritium evolution and tritium labeling of the blood do not completely coincide, since the lag in

¹The terms "exchangeable" or "labile" tritium or hydrogen refer to the dissociation of tritium or hydrogen atoms from the parent compound in the presence of water to form a hydrogen ion. Such an ionization allows an exchange of tritium with hydrogen ions of water. A compound such as hydrochloric acid undergoes a rapid "exchange" with water while methane will not "exchange" hydrogen ions with water due to lack of dissociation or ionization.

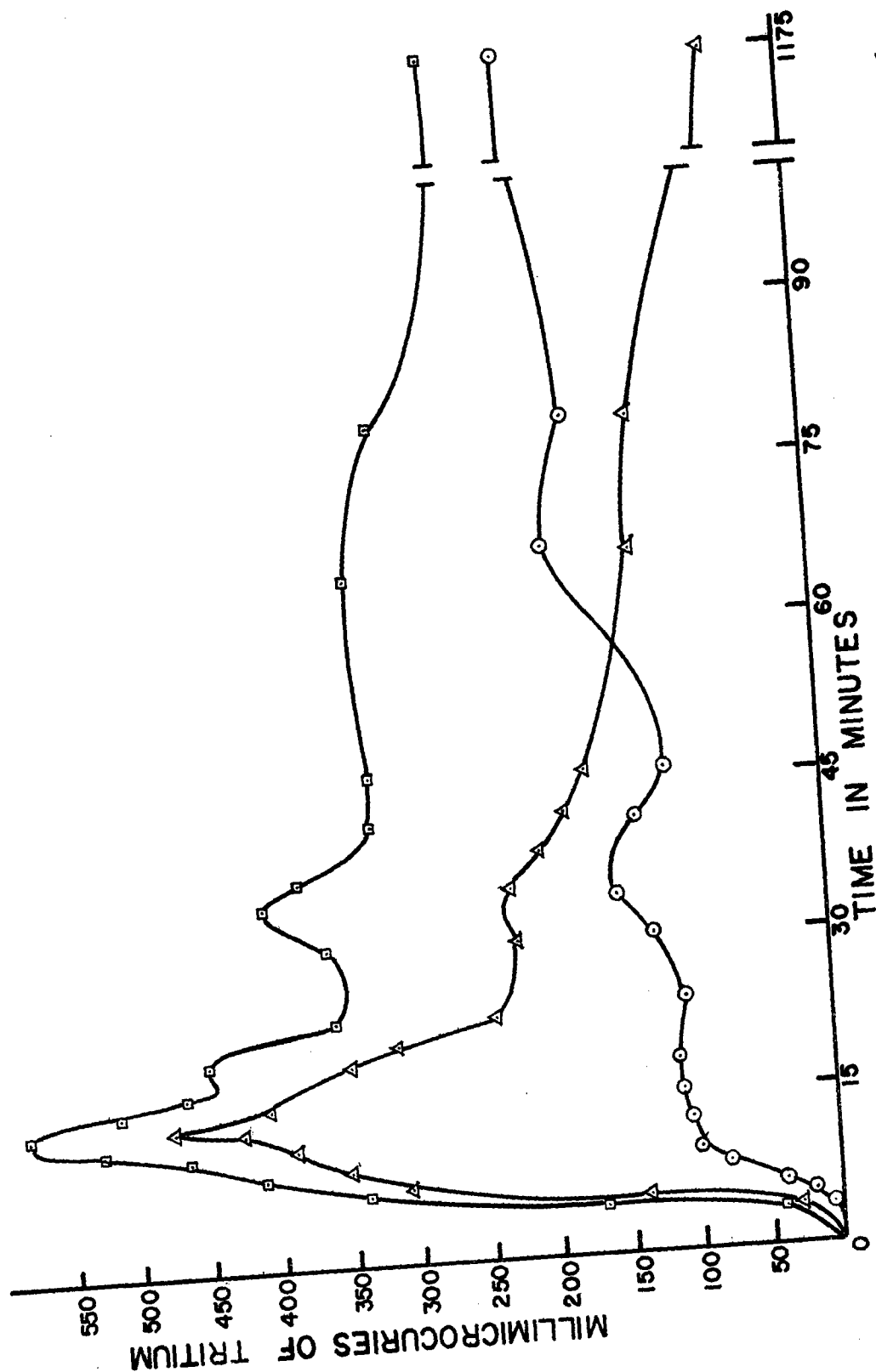


Figure 3. Tritium content per ml rabbit blood versus time in minutes after administration of B_5H_3 to an anesthetized rabbit. The symbols are as follows: \circ Tritium content in the water from one ml blood, Δ tritium content in one ml of dried blood, \square total tritium in water and dried blood from one ml of fresh blood.

TABLE I
IN VIVO CHANGES IN TRITIUM DISTRIBUTION IN RABBIT BLOOD
VS. TIME AFTER $B_5H_9^3$ ADMINISTRATION*

Time in Minutes After Administration	Millimicrocuries of Tritium			Ratio Solid/Water
	Dried Blood	Water	Total	
0.5	0	0	0	---
2.5	42	4	46	10.5
4	144	20	162	7.2
6	302	42	355	7.2
8	358	66	424	5.4
10	372	92	464	4.1
11	428	100	528	4.3
13	470	102	572	4.6
16	400	116	516	3.5
18	348	122	470	2.9
21	326	122	448	2.7
24	242	112	354	2.2
30	226	130	356	1.7
34	242	152	394	1.6
36	214	156	370	1.4
39	192	104	296	1.8
43	182	144	326	1.3
47	164	126	326	1.3
67	140	188	332	0.8
79	140	172	312	0.8
1,175 (19.6 hrs)	56	196	252	0.3

*Intraperitoneal administration of $3.7 \mu l B_5H_9^3$ containing $720 \mu c$ of tritium.
Rabbit weight was 840 grams.

response time of the animal respirometer was 5-6 minutes. Thus, as molecular tritium evolved from the rat, it did not appear in the flow ion chamber until approximately 5 minutes later. This was due largely to the moderate flow rate employed in relation to the sizeable volume of the animal chamber and drying column.

The results so obtained indicate that the transfer of tritium from pentaborane-9- H^3 to water in vivo occurred via a partial hydrolysis intermediate. It appears that for the first 20-30 minutes the non-volatile intermediate may have evolved some molecular tritium itself, since tritium recovery in water from blood was quite low initially. After the initial hydrolysis (30-40 minutes) little molecular tritium was formed and the partial hydrolysis product(s) was converted slowly, possibly enzymatically, to an intermediate(s) which allowed the tritium labeling to become ionizable and, subsequently, exchangeable with water. Essentially all of the tritium lost from the tritium-containing intermediate in dried blood after 34-40 minutes time can be accounted for in the water obtained upon lyophilization of the tritium labeled blood. The disappearance of tritium labeling per unit volume of blood, even up to 19 hours, was relatively slight (only 20% of the total), thus verifying that all of the pentaborane-9 may have undergone an initial reaction in less than 60 minutes.

Tritium Labeling Distribution in Rat Tissues and Blood Ten Minutes and Three Hours After $B_5H_9^3$ Administration

Experiments were carried out to show that a relatively large portion of the tritium labeling in the blood of rats during the initial hydrolysis of $B_5H_9^3$ was as an intermediate containing non-volatile and non-ionizable tritium atoms. A rat was sacrificed 10 minutes after $B_5H_9^3$ administration. The tritium labeling distribution in blood and various organs is shown in table II. It is readily apparent that virtually all of the tritium labeling was present in the dried residues of blood and liver tissues and not in water removed from them. Thus, the reactions of $B_5H_9^3$ in the rat appear to be similar, if not identical, to those in the rabbit.

The distribution of tritium labeling in tissues and organs of rats was examined after molecular tritium evolution from the administered $B_5H_9^3$ ceased (3 hours). The experiment was designed to establish the fate of the non-volatile tritium labeling incorporated into blood and liver of the rat. Thus, these experiments primarily were to determine the total incorporated tritium labeling in the various organs and tissues after molecular tritium was no longer being formed to any appreciable degree.

The distribution of tritium labeling in rats, 3 hours after administration of $B_5H_9^3$, is shown in table III. The amount of tritium radioactivity released as molecular tritium agreed very well with that obtained previously (figures 1 & 2). The remaining tritium labeling was distributed throughout the different tissues and organs of the rat rather uniformly. As can be seen in

TABLE II

TRITIUM DISTRIBUTION IN RAT TISSUES TEN MINUTES AFTER

ADMINISTRATION OF $B_5H_9^3$ *

Tissue	Fresh Wt. in Grams	Organ		$\mu\text{c}/\text{Total}$ Fresh Organ	$\mu\text{c}/\text{Gram}$ Dry Wt.	$\mu\text{c}/\text{ml}$ Organ Water	$\mu\text{c}/\text{Gram}$ Fresh Wt./mc Administered
		Dry Wt. in Grams	Water Vol. in ml				
Blood	17.5 [†]	3.2	14.0	106.3	31.9	0.3	7.1
Liver	8.0	3.4	4.6	34.2	9.5	0.4	5.0
Kidneys	1.6	0.4	1.0	1.7	1.5	1.1	0.1
Heart	0.9	0.2	0.6	0.6	0.9	0.7	0.1
Lungs	1.1	0.2	0.8	1.0	1.7	0.9	0.1
Spleen	0.8	0.2	0.6	1.0	2.0	1.0	0.2
Small Intestine	4.8	0.9	3.1	3.6	3.2	2.3	0.1
Large Intestine	2.3	0.5	1.8	3.8	1.3	1.7	0.2

* Intraperitoneal administration of $4.4 \mu\text{l } B_5H_9^3$ containing 850 μc of tritium.[†] Estimated value based upon blood weight being 7% of total body weight.

TABLE III
TRITIUM DISTRIBUTION IN RAT TISSUES THREE HOURS
AFTER ADMINISTRATION OF $B_5H_9^3$ *

Tissue	Fresh Weight Grams	μc /Gram		% of Total $B_5H_9^3$ Administered
		Fresh Weight/ mc of $B_5H_9^3$ Administered	μc /Total Organ	
Brain	2.0	0.8	0.6	0.2
Heart	1.0	1.1	0.4	0.1
Liver	12.8	2.1	10.2	2.7
Kidneys	2.5	1.6	1.5	0.4
Lungs	1.7	1.3	0.9	0.2
Stomach	2.2	2.1	1.8	0.5
Spleen	0.6	1.3	0.3	0.1
Gonads	3.7	1.1	1.5	0.4
Sm. Intestine	8.4	1.6	5.0	1.3
Lg. Intestine	1.9	1.6	1.1	0.3
Cecum	2.0	1.6	1.2	0.3
Pancreas & Mesentery Tissues	3.6	1.3	1.8	0.5
Fat	---	1.6	---	---
Muscle	---	0.8	---	---
Blood	---	1.3	13.5 [†]	3.6
Blood Plasma	---	1.3	---	---
Urine	---	---	9.3	2.5
Total tritium gas released from pentaborane-9- H^3 during the experiment			128	34%

[†]On a basis of blood being 7% of total body weight.

*Intraperitoneal administration of 1.9 μl $B_5H_9^3$ containing 370 μc of tritium.

table III, the level of tritium labeling in the liver and blood was much lower 3 hours after $B_5H_9^3$ administration, in comparison with that observed 10 minutes after $B_5H_9^3$ administration (table II). Thus, as in the rabbit, the bulk of the non-volatile tritium labeling becomes ionizable hydrogen atoms of water in the animal and hence, distributed throughout the tissues. If the total carcass mass is considered, the amount of tritium labeling distributed throughout various tissues can account for the majority of the tritium radioactivity which was not released as molecular tritium. Total muscle and fat mass was estimated to be 40 and 10% of total body weight respectively.

Tritium assays were carried out on certain of these labeled tissues and blood after three repeated lyophilizations. After each lyophilization, the tissues and blood were brought back to their original volume with distilled water. It was found that virtually all of the tritium labeling was in the water of the tissues and organs from rats which had been administered $B_5H_9^3$ 3 hours previously. To examine this aspect more closely, $B_5H_9^3$ was administered to two rats and, after 3 hours, blood samples were removed and assayed for tritium. The results are shown in table IV.

TABLE IV

TRITIUM DISTRIBUTION IN RAT BLOOD THREE HOURS AFTER I.P.

ADMINISTRATION OF $B_5H_9^3$

Time	Microcuries of Tritium per ml Blood per Millicurie of $B_5H_9^3$ Administered		
	Dried Blood	Water	Fresh Blood
Three Hours	0.25 ± 0.03	1.42 ± 0.1	1.67 ± 0.13

A much larger portion of the tritium labeling in blood was associated with the water of the blood from 3 hour exposed rats than with that from rats exposed for only 10 minutes. Thus, the amount of tritium in the dried blood solids 10 minutes after $B_5H_9^3$ administration was about 20 fold greater than in the water of blood. After 3 hours, the ratio reversed and the water then contained a 16 fold greater amount of tritium than the dried blood. This represents a total change in tritium distribution of greater than 300 fold. A similar change in the tritium labeling distribution in rabbit blood was observed (table I, figure 3).

The evidence presented indicates that pentaborane undergoes an initial hydrolysis losing a portion of its nine hydrogen atoms in intact animals to yield molecular hydrogen and giving rise to a partial-hydrolysis intermediate. The latter undergoes further hydrolysis in vivo relatively slowly

to alter the nature of the partial hydrolysis intermediate to form ionizeable hydrogen atoms which are exchangeable with water. The evidence strongly suggests that the type of hydrolysis or oxidation of the partial hydrolysis product may be enzymatic which allows the hydrogen to become exchangeable with, or actually form water in the blood.

Interaction of Pentaborane-9- H^3 with Blood In Vitro

The following experiments were carried out to establish the rate and extent of pentaborane-9- H^3 hydrolysis to intermediary compounds and hydrogen gas in the presence of blood. The flow schemes in figures 4 and 5 illustrate two types of processes employed for trichloroacetic acid (TCA) fractionation of blood which had been previously exposed to $B_5H_9^3$. Figure 4 shows the results of 5% TCA fractionation of blood which was dried by lyophilization after exposure to $B_5H_9^3$. It can be seen that the blood incorporated approximately 25-30% of the administered $B_5H_9^3$ radioactivity as a non-volatile tritium compound(s). Subsequently, treatment of the dried blood with 5% TCA resulted in extensive loss of tritium labeling while the precipitated protein fraction retained only 1-3% of the administered tritium. The water recovered in the first lyophilization contained virtually all of the ionizable tritium, which was 10% of the administered radioactivity (figure 4). Suspension of the dried blood in water and a second lyophilization resulted in 0.6% of the administered tritium exchanging or equilibrating into the water. A third lyophilization resulted in only 0.1% of the blood radioactivity exchanging into water. Combustion of the dried blood after the third lyophilization showed that 25-35% of the total tritium radioactivity administered was still retained in the dried blood residue. This tritium labeling would represent non-ionizeable tritium which would be associated with either carbon or boron.

Treatment of the dried blood- $B_5H_9^3$ reaction mixture, after lyophilization, with 5% TCA resulted in an extensive loss of molecular tritium which was in marked contrast to the small amount of tritium which was labile in the presence of water alone. Since the loss of tritium labeling was in the form of molecular tritium, it appears that a major portion of the non-exchangeable or non-labile tritium in blood after exposure to pentaborane-9- H^3 was present as an acid-labile compound with boron-to-hydrogen bonding. To examine further the acid-labile nature of the tritium compound(s) from $B_5H_9^3$ reaction with heparinized blood, exposures of blood to pentaborane-9- H^3 were made, as shown in figure 5. A TCA extraction was carried out in the cold immediately after exposure to $B_5H_9^3$ and before lyophilization. After TCA removal of the protein constituents, the TCA soluble fraction was subjected to a methanol-chloroform extraction to obtain the lipid constituents of blood. Since the lipid phase contained only 1% of the administered tritium (figure 5) while the aqueous phase contained 20%, the partial hydrolysis product must be associated with the aqueous phase rather than the lipid constituents of blood. However, it must be noted that the partial hydrolysis product continued to hydrolyze during the methyl alcohol-chloroform extraction procedure, as evidenced by the continued loss of molecular

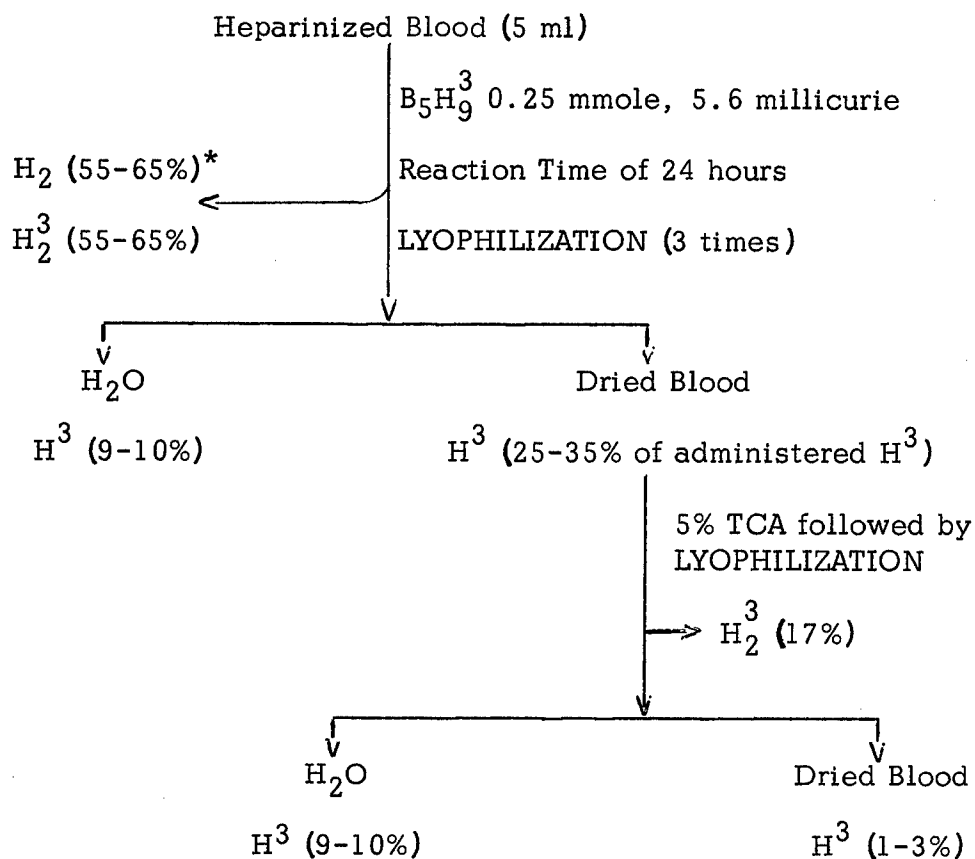


Figure 4. Flow scheme for the TCA fractionation of blood which has been exposed to pentaborane-9- H^3 . All recoveries are expressed as percent of tritium administered as pentaborane-9- H^3 . Trichloroacetic acid (TCA) treatment after lyophilization.

*Based upon pentaborane-9 concentration and a theoretical yield of 12 moles H_2 per mole of pentaborane-9.

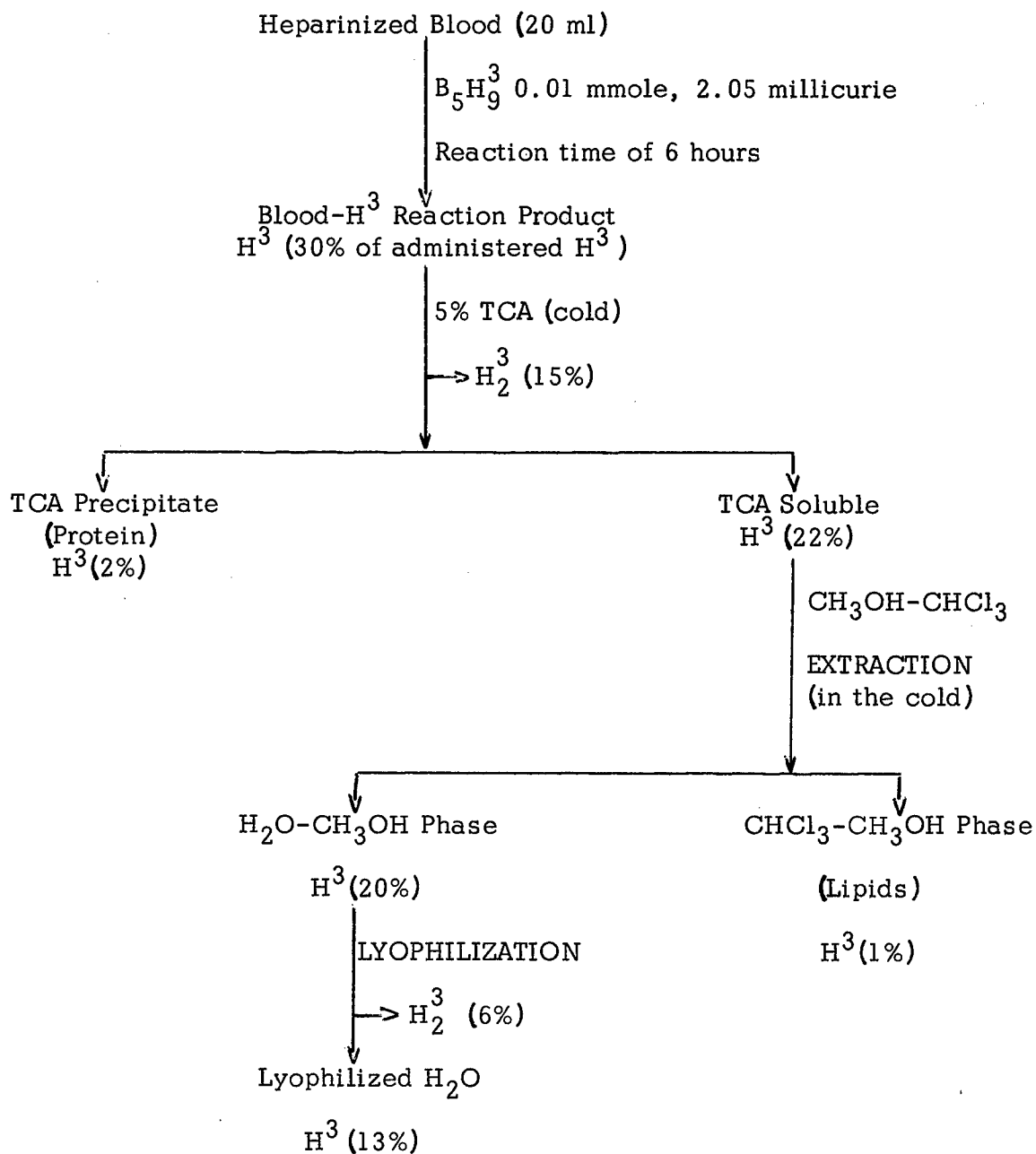


Figure 5. Flow scheme for the TCA fractionation of blood which has been exposed to pentaborane-9- H^3 . All recoveries are expressed as percent of tritium administered as pentaborane-9- H^3 . Trichloroacetic acid (TCA) treatment without prior lyophilization.

tritium during the fractionation. From this evidence it must be concluded that the partial hydrolysis product is relatively sensitive to both TCA and the solvents employed. Similarly, and as shown below, the partial hydrolysis product from B_5H_9 and L-histidine reactions was found to be very acid sensitive and contained B-H bonding.

Hydrolysis of Pentaborane-9- H^3

The hydrolysis of pentaborane-9- H^3 in various hydrolyzing media was carried out and the results shown in table V. Only a small amount of tritium labeling was retained in the hydrolyzing medium from the hydrolysis reactions catalyzed by ethanol or dioxane. The amount of tritium labeling exchangeable into water and retained in water, upon lyophilization, was found not to exceed 13%. When the hydrolysis was catalyzed by DL-alanine, less than 1% of tritium labeling was even found in the hydrolysis solvent, water.

A very interesting aspect of the nitrogen-catalyzed hydrolysis of $B_5H_9^3$ was the ability of 0.2 M L-histidine to cause the formation of a partial hydrolysis product. This non-volatile intermediate retained as much as 36% of the total tritium labeling derived from pentaborane-9- H^3 . The tritium labeling present was not labile in the presence of water but could be released very rapidly as molecular tritium by dilute acid treatment. Since the yield of partial-hydrolysis product was dependent upon L-histidine concentration it appears that this intermediate(s) is an anion complexed with histidine and contains B-H bonding. The nearly complete hydrolysis as pentaborane-9- H^3 in the presence of a lower concentration of L-histidine (0.02 M) indicates that L-histidine can catalyze the hydrolysis as well as stabilize a partial hydrolysis product. All of the non-volatile intermediates formed with blood, bovine serum albumin, niacinamide and L-histidine were found to release molecular tritium upon treatment with dilute acid. This fact indicates that essentially all of the tritium in these complexes is present as B- H^3 bonding and not as C- H^3 bonding. If the pentaborane-9- H^3 had caused chemical reduction of these compounds, a portion of the tritium incorporated could be exchangeable with water but would not be acid labile to form molecular tritium.

It should be mentioned that the rate of hydrolysis in vacuo cannot be compared with that observed in the in vivo experiments, even though the initial phase of hydrolysis may have a similar mechanism. The nitrogen compounds catalyzed a rapid hydrolysis of pentaborane-9 as did dioxane and ethanol, while the rate of hydrolysis in the presence of water alone is extremely slow (ref. 21).

TABLE V
HYDROLYSIS OF PENTABORANE-9-H³

Hydrolysis Medium	B ₅ H ₉ Concen. in mmole	Reaction Time Hours	% Yield H ₂ Gas	% H ³ in Reaction Residue*	% H ³ in Reaction Solvent After Lyophilization
1 ml of 50% dioxane-water	0.44	16	93		13
20 ml 50% water-alcohol					9
		3	21		
		24	58		
4 ml fresh rat blood	0.23	72	73	14	9
Bovine serum albumin 500 mg in 5 ml H ₂ O	0.31	3	53	10	9
DL-alanine 2 mmole in 5 ml water	0.32	16	93		<1
L-histidine (free base) 0.1 mmole in 5 ml water	0.38	21	90		
L-histidine (free base) 0.6 mmole in 5 ml water	0.32	24	55		
L-histidine (free base) 1.0 mmole in 5 ml water	0.35	16	32	33	
L-histidine (free base) 1.0 mmole in 5 ml water	0.36	16	40	36	
Niacinamide 2 mmole in 5 ml water	0.33	8	69	10	
Imidazole 0.3 mmole in 5 ml water	0.32	4	74		
Imidazole 0.6 mmole in 5 ml water	0.30	4	74	<1	
Imidazole 2.0 mmole in 5 ml water	0.30	11	73		

*The percent H³ in the reaction residues is the percent of H³ which did not volatilize upon lyophilization of the reaction mixture at the end of the reaction period. The percent H³ was based upon percent H³ administered as pentaborane-9-H³.

SECTION V

CONCLUSIONS

The mechanism of toxicity of pentaborane-9 is still unknown. From the work presented, it is now known that pentaborane-9 upon administration to mammalian animals is rapidly hydrolyzed to yield both molecular hydrogen and a transitory intermediate. This intermediate(s) is present mainly in the blood and liver of B_5H_9 intoxicated animals. While this rather labile intermediate has not been identified, some of its properties have been examined. The product is non-volatile and yields molecular hydrogen upon being treated with acids. Thus, the intermediate appears to retain a portion of the boron to hydrogen bonding of pentaborane-9.

The fate of the partial hydrolysis intermediate formed in vivo is uncertain. However, evidence presented indicates that the intermediate decreases in concentration rather slowly and without the formation of additional molecular hydrogen. All of the tritium labeling in the intermediate appears to become exchangeable with ionizable hydrogen atoms of body water.

The hydrolysis of pentaborane-9 as catalyzed by blood, dioxane, ethanol and certain nitrogen compounds is relatively rapid with evolution of molecular hydrogen to a variable degree. The reaction of pentaborane-9 with blood or L-histidine or to some extent niacinamide resulted in the formation of a stable partial-hydrolysis product. This hydrolysis product has many of the properties of an intermediate formed in vivo from B_5H_9 . The hydrolysis of pentaborane-9 in the presence of varying amounts of L-histidine indicates that the intermediate formed depends upon the presence of L-histidine for stability since a lower concentration of L-histidine (0.02 M) relative to pentaborane (0.3 mmole) resulted in almost complete hydrolysis of pentaborane-9 as measured by molecular hydrogen production.

Dilute acids caused destruction of the partial hydrolysis intermediate of $B_5H_9^3$ and formation of almost the theoretical yield of molecular tritium. However, the disappearance of the $B_5H_9^3$ reaction intermediate in vivo may be enzymatic since no appreciable molecular hydrogen evolution occurred during its disappearance, and the appearance of an approximately equivalent amount of tritium labeling in blood and tissue water.

REFERENCES

1. Dost, F. N., D. J. Reed and C. H. Wang, Lethality of Pentaborane-9 in Mammalian Animals, Aerospace Medical Research Laboratories Technical Documentary Report AMRL-TDR-63-128, Wright-Patterson Air Force Base, Ohio, 1963.
2. Hill, William H., George J. Levinskas and Jean M. Merrill, "Correlation of the Reducing Power of Boranes with Their Toxicity," A.M.A. Arch. Ind. Health, Vol 17, pp 124-128, 1958.
3. Jacobson, K. H., Toxicity of Borane Fuels, Trans. Symposium on Health Hazards of Military Chemicals, p 55, 1958.
4. Levinskas, G. J., The Toxicology of Boron Hydrides, Chemical Warfare Laboratories Report CWL-TM-27-7, Army Chemical Center, Maryland, December, 1956.
5. Levinskas, G. J., M. R. Paslian and R. Bleckman, "Chronic Toxicity of Pentaborane Vapor," Am. Ind. Hyg. Assn. J., Vol 19, p 46, 1958.
6. Lowe, H. J. and Gustave Freeman, "Boron Hydride (Borane) Intoxication in Man," A.M.A. Arch. Ind. Health, Vol 16, p 523, 1957.
7. Miller, D. F., A. Tamas, L. Robinson and E. Merriweather, "Observations on Experimental Boron Hydride Exposures," Toxicol. Appl. Pharm., Vol 2, p 430, 1960.
8. Schoettlin, C. E., G. M. Cianko, R. D. Walter and T. Freedman, Toxicological Research on Central Nervous System Effects of Boron Fuels, ASD Technical Report 61-438, Aerospace Medical Laboratory Wright-Patterson Air Force Base, Ohio, September 1961.
9. Svirebely, J. L., "Subacute Toxicity Studies of Decaborane and Pentaborane Vapors," A.M.A. Arch. Ind. Health, Vol 10, p 305, 1954.
10. Weatherby, J. H., Determination of Pentaborane in Air and the Symptoms and Treatment of Pentaborane Poisoning in Animals, Army Chemical Warfare Laboratories Special Publication 2-5, Army Chemical Center, Maryland, 1957.
11. Weir, F. W., D. W. Bath and M. H. Weeks, Short-Term Inhalation Exposures of Rodents to Pentaborane-9, Aeronautical Systems Division Technical Report 61-663, Wright-Patterson Air Force Base, Ohio, 1961.

12. Weir, F. W., V. M. Seabaugh, M. M. Mershon, D. G. Burke and M. H. Weeks, Short-Exposure Inhalation Toxicity of Pentaborane in Dogs, Aerospace Medical Research Laboratories Technical Documentary Report 62-145, Wright-Patterson Air Force Base, Ohio, 1962.
13. Weir, F. W., V. M. Seabaugh, M. M. Mershon, D. G. Burke and M. H. Weeks, "Short-Exposure Inhalation Toxicity of Pentaborane in Animals," Toxicol. Appl. Pharm., Vol 6, pp 121-131, 1964.
14. Weiss, H. G. and I. Shapiro, "Diborane from the Sodium Borohydride-Sulfuric Acid Reaction," J. Amer. Chem. Soc., Vol 81, p 6167, 1959.
15. McCarthy, L. V. and P. A. Di Giorgio, "The Pyrolysis of Diborane and the Synthesis of Pentaborane," J. Amer. Chem. Soc., Vol 73, p 3138, 1951.
16. Owen, A. J., "The Pyrolysis of Diborane," J. Appl. Chem., Vol 10, pp 483-493, 1961.
17. Kelly, R. L., E. A. Peets, S. Gordon and D. A. Buyske, "Determination of C^{14} and H^3 in Biological Samples by Schöniger Combustion and Liquid Scintillation Techniques," Anal. Biochem., Vol 2, pp 267-273, 1961.
18. Sheppard, H. and W. Rodegker, "Determination of H^3 and C^{14} in Biological Materials Using Oxygen-Bomb Combustion," Anal. Biochem., Vol 4, pp 246-251, 1962.
19. Dost, F. N., D. J. Reed and C. H. Wang, Fate of UDMH and MMH in Rats, Aerospace Medical Research Laboratories Report No. AMRL-TR-64-111, Wright-Patterson Air Force Base, Ohio, November 1964.
20. Bligh, E. G. and W. J. Dyer, "A Rapid Method of Total Lipid Extraction and Purification," Can. J. Biochem. and Physiol., Vol 37, pp 911-917, 1959.
21. Butler, G. W., T. D. Parsons, C. H. Wang and D. J. Reed, Vapor Phase Hydrolysis of Pentaborane-9, Aerospace Medical Research Laboratories Technical Documentary Report AMRL-TDR-63-129, Wright-Patterson Air Force Base, Ohio, 1963.

THE EFFECT OF PENTABORANE-9 UPON GLUCOSE METABOLISM IN RATS

SECTION I

INTRODUCTION

Pentaborane-9 is known to be quite toxic (ref. 1 & 2) and to cause hyperactivity of the central nervous system resulting in convulsions and death in mammals (ref. 2). An extensive loss of molecular hydrogen from B_5H_9 intoxicated animals has been shown to occur. The accompanying reducing power of B_5H_9 could result in a possible impairment of electron transport systems which undergo oxidation-reduction via co-factors such as pyridine nucleotides and flavins. Such co-factors are known to be readily reduced by other reducing agents, i.e., sodium borohydride.

Decaborane and HEF-3 treated rabbits show a prolonged glucose tolerance curve (ref. 3). In addition, liver glycogen was found to be depleted. Xanthine oxidase, a flavoprotein, was found to be inhibited 80-90% by intoxication of mice with decaborane (ref. 4).

The present report describes experiments on the effect of pentaborane-9 upon glucose catabolism in rats. The radiorespirometric technique of Wang et al (ref. 5) was employed.

SECTION II

MATERIALS

Experimental Animals

All studies described in this report were conducted with male Sprague-Dawley rats obtained from the Northwest Rodent Company, Pullman, Washington. The rats were maintained on Purina Laboratory Chow and water ad libitum until 48 hours prior to the experiments. During the 48 hour period prior to the experiments the rats received water only. The rats weighed 250-260 grams at the start of the experiments and after fasting for 48 hours.

Chemicals

The pentaborane-9 used in the present study was manufactured by the Callery Chemical Company, Callery, Pennsylvania. It was handled by the procedures previously described elsewhere (ref. 1).

Radiochemicals

Glucose-1- C^{14} , -2- C^{14} and -6- C^{14} (sp.act. 1 mc/mmole) were

supplied by New England Nuclear Corporation, Boston, Massachusetts. Glucose-3,4- C^{14} (sp.act. 60 μ c/mmole) was prepared in this laboratory by the method of Wood et al (ref. 6).

SECTION III

METHODS

The radiorespirometer employed in this study has been described in detail in a previous report (ref. 7).

Pentaborane-9 Administration

Pentaborane-9 was administered intraperitoneally by microsyringe injection at the beginning of an experiment. Each animal was given 2.5 mg/kg of pentaborane-9.

Radiorespirometry Experiments

Each fasted rat was administered via stomach tube 1.5 grams of glucose dissolved in 2.5 ml water containing 2 μ c of either glucose-1- C^{14} , -2- C^{14} , -3,4- C^{14} or -6- C^{14} at the start of an experiment. The animals were then placed in the radiorespirometric apparatus. A flow rate of one liter per minute of CO_2 free air into the respiration chamber was maintained during the experiments. The respiratory $C^{14}O_2$ was trapped in 2N NaOH (CO_2 free) and assayed in a manner described previously (ref. 7).

SECTION IV

RESULTS AND DISCUSSION

Total Carbon Dioxide Production

The production of respiratory CO_2 by rats was essentially unaffected by pentaborane-9 intoxication at a dose level of 2.5 mg/kg as shown in table I. While a slight reduction in CO_2 output occurred during the first 1-2 hours after intoxication, the total output after 16 hours was not inhibited and may have been actually stimulated somewhat.

$C^{14}O_2$ Formation from Specifically Labeled Glucoses

Control Rats

The $C^{14}O_2$ recoveries from 48 hour fasted rats, metabolizing C^{14} specifically labeled glucose substrates, are shown in table II in 4 hour intervals.

TABLE I

TOTAL CARBON DIOXIDE FORMATION BY PENTABORANE INTOXICATED RATS

Millimoles of CO ₂ Carbon in Sixteen Hours		
Experiment	Controls	Intoxicated Rats 2.5 mg pentaborane/kg
Glucose-1-C ¹⁴ *	129 125 ⁺	140 162
Glucose-2-C ¹⁴	127 167	146 174
Glucose-3,4-C ¹⁴	122 156	155 161
Glucose-6-C ¹⁴	131 138	150
Average	137	155

*C¹⁴ labeled substrates are shown only to identify the individual experiments and have no bearing on total respiratory CO₂ formed.

⁺Individual values are presented for the duplicate sets of experiments.

TABLE II

PERCENT CUMULATIVE RECOVERY OF C¹⁴O₂ FROM RATS METABOLIZING
SPECIFICALLY LABELED GLUCOSE*

Controls					Pentaborane Treated (2.5 mg/kg body weight)			
Time	C ¹⁴ Labeled Carbon Atoms of Glucose							
Hours	C-1	C-2	C-3,4	C-6	C-1	C-2	C-3,4	C-6
4	16	18	22	15	14	13	12	8
8	36	31	41	30	27	31	28	21
12	43	37	47	38	34	38	33	30
16	48	42	51	45	39	44	36	36

*The values presented are average values of duplicate experiments.

The data presented gives evidence that the glycolytic (EMP) pathway plays a dominant role in glucose catabolism to form respiratory CO_2 . This is clearly indicated by the prompt and extensive recovery of C-3 and C-4 of glucose in respiratory CO_2 . Also, it can be seen that both C-6 and C-1 of glucose were converted to respiratory CO_2 as extensively as C-2 of glucose. This fact suggests that pathways other than the EMP route were operative in the utilization of glucose by the rat.

Preferential conversion of glucose C-1 to respiratory CO_2 in comparison to C-2 indicates the operation of the pentose phosphate (PP) pathway. This pathway has been demonstrated in the rat by the work of Spiro and Ball (ref. 8).

The conversion of C-6 of glucose to CO_2 to a slightly greater extent than C-2 presumably reflects the operation of a preferential decarboxylation pathway for the oxidation of C-6, such as the glucuronate pathway (ref. 9). Along this pathway glucose is thought to be oxidized to glucuronic acid which in turn is decarboxylated to form CO_2 from C-6 and a pentose. The pentose unit is then converted to reformed hexose for further metabolism.

Intoxicated Rats

The recoveries of C^{14}O_2 from specifically labeled glucoses being metabolized by B_5H_9 intoxicated rats are shown in table II. The effect of the administered pentaborane upon glucose catabolism occurred primarily during the first 4-hour period of the experiments. Thus, the conversion of C-3, 4 and C-6 of glucose to respiratory CO_2 in the intoxicated rats was strongly inhibited while the recovery from C-1 was only slightly inhibited. From this, it appears that the EMP pathway for glucose catabolism may have been inhibited in rats to a considerable degree. However, inasmuch as C-1 and C-6 of glucose are catabolized to CO_2 in an identical manner via the glycolytic pathway; one would expect that the C^{14} yield from C-1 reduced to the same degree as the C^{14}O_2 yield from C-6 during the first 4 hours. This is not so. In fact, one finds that C^{14}O_2 from C-1 was only slightly decreased in the intoxicated rats. It can, therefore, be concluded that the alternate pathway for the conversion of C-1 to CO_2 , such as the pentose phosphate pathway, may have been stimulated to some extent to account for the observed extent of CO_2 formation from C-1 of glucose. The observed reduction of the conversion of C-2 to respiratory CO_2 during the first 4-hour period is also in line with the suggested inhibition of glycolysis. From the C^{14}O_2 yields observed in the glucose-3, 4- C^{14} and glucose-6- C^{14} experiments, it can be assumed that any major effect pentaborane-9 has upon the operation of the glycolytic pathway occurred during the first 4-hour interval.

During the 4-8 hour period of the experiment the conversion of C-1 of glucose to respiratory CO_2 was inhibited more extensively than that observed in the glucose-3, 4- C^{14} and glucose-6- C^{14} experiments. This may indicate that the pentose phosphate pathway was also inhibited to some extent by pentaborane-9 although the response was somewhat slower.

Total respiratory CO_2 formation was slightly increased which may reflect, in part, a hyperglycemic response to pentaborane-9 treatment similar to that demonstrated for decaborane and its derivative HEF-3 (ref. 3 & 6).

SECTION V

SUMMARY

It has been possible to demonstrate alterations of carbohydrate metabolism in rats resulting from pentaborane-9 intoxication. While these effects may reflect in part, hyperglycemia, glycolysis appears to be inhibited and the pentose phosphate pathway increased slightly during the first 4-hour interval. During the second 4-hour interval, the pentose phosphate pathway may have been inhibited to a considerable degree while glycolysis was returning to a rate approaching that observed with the control rats.

Total CO_2 formation by B_5H_9 intoxicated rats during a 16-hour period after intoxication was slightly stimulated.

REFERENCES

1. Dost, F. N., D. J. Reed and C. H. Wang, Lethality of Pentaborane-9 in Mammalian Animals, 6570th Aerospace Medical Research Laboratories, Report No. AMRL-TDR-63-128, Wright-Patterson Air Force Base, Ohio, December, 1963.
2. Weir, Francis, W., Van M. Seabaugh, M. M. Mershon, D. G. Burke, and M. H. Weeks, "Short-Exposure Inhalation Toxicity of Pentaborane in Animals," Toxicolog. Appl. Pharmacol., Vol 6, pp 121-131, 1964.
3. Tamas, A. A., State-of-the-Art Report on Health Hazards of Borane Fuels and Their Control, Aero Medical Laboratory, Wright-Patterson Air Force Base, Ohio, October, 1958.
4. Hill, David L., Some Aspects of the Chemistry and Biochemistry of Decaborane, CWL Special Publication 2-15, U. S. Army Chemical Warfare Laboratories, Army Chemical Center, Maryland, 1958.
5. Wang, Chih H., L. P. Snipper, Oya Eilen and Betty Hawthorne, "Catabolism of Glucose and Gluconate in Intact Rats," Proceedings of the Society for Experimental Biology and Medicine, Vol 111, pp 93-97, 1962.
6. Wood, H. G., N. Lifson and V. Lorber, "The Position of Fixed Carbon in Glucose from Rat Liver Glycogen," Journal of Biological Chemistry, Vol 159, pp 475-489, 1945.
7. Reed, D. J., F. N. Dost, R. S. McCutcheon, R. D. Barbour and C. H. Wang, Biochemical and Pharmacological Studies of 1,1-Dimethylhydrazine, 6570th Aerospace Medical Research Laboratories, Report No. AMRL-TDR-63-127, Wright-Patterson Air Force Base, Ohio, December, 1963.
8. Spiro, Mary Jane and Eric G. Ball, "A Comparison of the Pathways of Glucose Catabolism in the Normal and Hyperthyroid Rat," Journal of Biological Chemistry, Vol 231, pp 31-40, 1958.
9. Eisenberg, Frank, Jr., Peter G. Dayton and J. J. Burns, "Studies on the Glucuronic Acid Pathway of Glucose Metabolism," Journal of Biological Chemistry, Vol 234, pp 250-253, 1959.

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14. KEY WORDS	LINK A		LINK B		LINK C	
	ROLE	WT	ROLE	WT	ROLE	WT
Pentaborane-9-H ³ metabolism						
Metabolites						
Tritium distribution						
H ₂ ³ Respiration						
Rats						
Rabbits						
Pentaborane-9 intoxication						
C ¹⁴ -Glucose metabolism						
C ¹⁴ O ₂ Respiration						

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